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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : G01N 33/574, 33/53, C07K 15/28 | A1

(11) International Publication Number: WO 95/02188

(43) International Publication Date: 19 January 1995 (19.01.95)

(21) International Application Number: PCT/US93/06579

(22) International Filing Date: 12 July 1993 (12.07.93)

(30) Priority Data:
Not furnished 9 July 1993 (09.07.93) US

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(81) Designated State: CA.

Published
With international search report.

(54) Title: ASSAY FOR YKL-40 AS A MARKER FOR DEGRADATION OF MAMMALIAN CONNECTIVE TISSUE MATRICES

(57) Abstract

The invention is a method of identifying the presence of a disease state in a mammal which is associated with degradation of connective tissue in the mammal which contains the protein known as YKL-40. The method is a competitive immunoassay for YKL-40. It can be used, for example, to identify the presence of inflammatory or degenerative joint disease and tumor metastasis (to the extent it can be correlated to serum YKL-40 levels). Serum YKL-40 levels as detected and quantified by the inventive method are also suggestive of the prognosis for the length of survival in breast cancer patients following recurrence and/or metastasis of their cancers.

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ASSAY FOR YKL-40 AS A MARKER FOR DEGRADATION OF MAMMALIAN CONNECTIVE TISSUE MATRICES

STATEMENT OF GOVERNMENT RIGHTS

This invention was made in part with Government support under Grant No. AR-27029, awarded by the National Institute of Health. The Government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

1. *Field of the Invention*

The invention relates to the identification of a circulating protein by-product of extracellular fiber matrix metabolism in mammalian connective tissues. More specifically, it is directed to assays for the detection and quantification of YKL-40, a protein by-product of connective tissue metabolism in mammals. It also involves correlating serum levels of YKL-40 in a mammal to the presence and status of diseases in which matrix metabolism plays a role, such as joint disorders and the metastasis of certain tumors.

2. *Description of Related Art*

The extracellular matrix of mammalian connective tissues (such as articular cartilage of joints and vascular wall tissue of the vascular and lymphatic system) provides (to varying degrees) a barrier to the migration of cells from the tissue and strength to it. In certain disease processes, however, the matrix is d graded by hydrolytic enzymes. As the matrix d grades, the integrity of the tissue is impaired, which may allow tissue cells and by-products of the matrix metabolism to escape into bodily fluids and/or lymphatic or vascular

circulation. Detection of these molecules and cells can, in certain instances, provide information regarding the biochemical characteristics of the extracellular matrix, including how it is synthesized and how it is lost. Also, where a particular by-product of abnormal matrix metabolism is closely related to a disease process, quantification of that by-product in the patient's body fluids and/or tissues can help clinicians track the progress of the disease.

For example, human joint cartilage is known to contain several different types of proteins and proteoglycans, a few of which are present only in cartilage. These matrix constituents are released from cartilage tissue as it degrades during the course of certain joint diseases. The quantity of released matrix constituents (including fragments thereof and related macromolecules) present in a particular fluid or tissue may correlate to the intensity of the disease. Conversely, where the damage to the cartilage is reversible (as in secondary reactive arthritis caused by infection of the joint tissue), a reduction in levels of previously measured released matrix constituents may correlate to a degree of remission of the disease.

In practice, however, identification of reliable markers for metabolism of cartilage and other connective tissues and development of assays for them has proved to be a difficult task. Certain released fragments and molecules may be rapidly cleared from circulation by the lymph nodes, liver and phagocytosis (see, e.g., Frazer, *et al., Hyaluronan: Sources, Turnover and Metabolism,* Clinical Impact of Bone and Connective Tissue Markers 31-49 (Acad. Press, 1989); Smedsrod, "Catabolism in Liver Sinusoids", id. at 51-73; and, Heinegard, *et al., Brit. J. Rheumatol.*, 30 (Suppl. 1): 21-24, 1991). Further, certain molecules are present in several different connective tissues, thus making correlation to metabolism in a particular tissue based on circulating levels of the molecule uncertain. Even where levels of a particular molecule can be traced to metabolism in the tissue of interest, the molecules may decline to

undetectable levels or be biochemically altered in structure during those stages of a disease when a substantial quantity or connective tissue has been lost.

Not surprisingly, therefore, attempts to develop serum assays to relate levels of certain proteins to joint disease activity have met with mixed success. Rohde and co-workers have described radioimmunoassays for serum levels of amino-terminal type III procollagen peptide and its degradation products in rheumatoid arthritis (RA) patients (Rohde, *et al. Eur. J. Clin. Invest.*, 9:451-459, 1979). This propeptide (P-III-NP) can be detected in several body fluids; a subsequent report attempted to correlate serum levels of P-III-NP to disease activity using the Rhode, *et al.* radioimmunoassay (Hørslev-Petersen, *et al., Arth. and Rheum.*, 5:592-599, 1986). While the concentrations of serum P-III-NP were significantly elevated in patients with active RA, these concentrations were also elevated to a similar degree in patient's with inactive RA, thus making distinction between the two states based on P-III-NP levels alone difficult.

Assays of serum levels of other connective tissue metabolites in RA patients have been attempted in connection with treatment protocols to gauge the success of those protocols, again with mixed success. For example, Hrslev-Petersen, *et al.*, measured serum levels of P-III-NP, immunoreactive propyl 4-hydroxylase protein (1RPH), 7S domain of collagen type IV (Col IV, 7S) and fragment PI of laminin (S-Lam), which are associated with metabolism of extracellular interstitial collagens and basement membranes.

Although serum levels of P-III-NP, 1RPH and Col IV, 7S were elevated in RA patients (as compared with healthy adults), the levels did not decline to normal ven with apparent remission of the disease. Also, levels of S-Lam remained at normal in both active and inactive RA patients. As a result, th presence and quantity of these proteins in serum does not appear to clearly correlate to the progress or remission of RA.

Similar difficulties are presented in identifying reliable markers for the progress of other connective tissue diseases. Identification of candidate molecules and fragments which may serve as reliable markers for connective tissue metabolism is, therefore, an important goal of clinical chemistry research. To this end, the expression of given proteins by matrix-forming cells has been assessed by immunologic assays for antigen and by cDNA hybridization assays for mRNA. Isolation of proteins from the extracellular matrix is, however, limited to the identification of secreted proteins that become abundant constituents of that matrix. As a result, identification of candidate proteins has been limited.

In 1992, the inventors described a method for identification of all proteins secreted by a matrix-forming cell (Johansen, *et al., J. Bone and Min. Res.*, 7:501-512, 1992). Using this method, a 40kD protein was identified as a secreted protein of human bone cells. The inventors hypothesized that the protein (named YKL-40 after its first three amino acids at the N-terminus and its molecular weight) could play a role in the action of Vitamin D in bone.

As described in detail below, it has since been discovered that YKL-40 can serve as a reliable marker for joint disease. Surprisingly, it has also been discovered that serum levels of YKL-40 are also substantially elevated in patients with metastasis of breast cancer cells, particularly those patients who survive for a relatively short period of time following recurrence and metastasis of their cancer. The methods for detecting and quantifying levels of YKL-40 in biological samples described herein, therefore, provide a means of charting the progress of not only joint disease, but also cancer cell metastasis. Further, based on the apparent relationship of serum levels of YKL-40 to connective tissue metabolism, it can be reasonably pr dicted that the methods described will be of use in the diagnosis and monitoring of other diseases in which connective tissue metabolism plays a role, such as osteoporosis.

SUMMARY OF THE INVENTION

Detection and quantification of a marker for diseases whose activity can be correlated to loss and/or synthesis of connective tissue matrices can be of value in diagnosing and monitoring the progress of both the disease and its amelioration. One such marker is YKL-40, a protein of about 40kD molecular weight which has been found in elevated concentrations in the blood and synovial fluid of human patients with joint disease as well as in the blood of human patients with breast cancer.

A point of commonality between these two conditions is their relationship to connective tissue loss. Specifically, connective tissue loss in joint disease results from its degradation by enzymes released in the disease process. In cancer cell metastasis, it is believed that degradation of the connective tissue of vessel walls and, possibly, of body organs permits migration of cells from the primary cancer tissue.

An object of the invention, therefore, is to provide a method of detecting and quantifying YKL-40 in biological samples using a detectably labelled antibody specific for YKL-40 or detectably labelled antigen (YKL-40).

Another object of the invention is to provide methods for diagnosis of diseases whose activity can be correlated to the loss and/or synthesis of connective tissue as indicated by levels of YKL-40 detected in a biological sample. In this respect, the invention is expected to be of particular use in the diagnosis of joint disease (such as RA), of cancer cell metastasis (as in, for example, breast cancer) and of diseases related to loss of connective tissue in bon such as osteoporosis.

Another object of the invention is to provide methods for the quantification of levels of YKL-40 to track the progress of a disease and/or its amelioration which is associated with metabolism of connective tissue containing YKL-40. Again, the invention is expected to be of particular use in tracking the progress and/or amelioration of joint disease (such as RA), of cancer cell metastasis (as in, for example, breast cancer) and of diseases related to the loss of connective tissue in bone such as osteoporosis.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows the elution position of substantially pure serum YKL-40 on a gel filtration column.

FIGURE 2 depicts the results of a radioimmunoassay for YKL-40 in biological samples (serum and synorial fluid) taken from human patients with inflammatory rheumatic joint disease. Purified YKL-40 is indicated by •. Serum levels of YKL-40 in a healthy person is indicated by ○; in serum from a rheumatoid arthritis patient by ▲, and in synovial fluid of a rheumatoid arthritis patient by □.

FIGURE 3 depicts the results of assays for levels of YKL-40 and other biochemical markers of joint disease in serum taken from human patients diagnosed as having joint disease.

FIGURE 4 depicts changes in serum YKL-40 levels in patients with active RA before and after treatment with methylprednisolone (MP), (indicated by ○), and in a control group which received a placebo (indicated by •).

FIGURE 5 (a)-(b) depicts and correlates in serum YKL-40 levels in the patients described with respect to FIGURE 4 with in their serum levels of other biochemical markers of joint disease.

FIGURE 6 shows a Kaplain-Meier survival curve, which relates the serum levels of YKL-40 measured in 60 breast cancer patients (aged 29-78 years) following recurrence and metastasis of their cancers to the length of time that each pati nt subs quently surviv d.

FIGURE 7 depicts serum levels of YKL-40 in 137 disease-free women, aged 20-79 years.

FIGURE 8 is a graph which identifies the serum levels of YKL-40 in breast cancer patients (measured as described with respect to FIGURE 6) and shows if and when each patient subsequently died as a result of their illness.

FIGURE 9 is a graph which relates the serum levels of YKL-40 in breast cancer patients (measured as described with respect to FIGURE 6) with the presence of metastasis to bone in each patient as diagnosed by x-ray evaluation.

FIGURE 10 is a graph which relates the serum levels of YKL-40 measured as described with respect to FIGURE 6 with the presence of metastasis to bone in each patient as diagnosed by bone marrow biopsies.

DETAILED DESCRIPTION OF THE INVENTION

A. Definitions

The following definitions are provided to simplify discussion of the invention. They should not, therefore, be construed as limiting the invention, which is defined in scope by the appended claims.

1. "Antibody" includes intact molecules as well as fragments thereof such as Fab and $F(ab')_2$ which are capable of binding an epitopic determinant on the YKL-40 protein.

2. "Antigen" (as used in the context of the inventive assay) refers to the YKL-40 protein or fragments thereof. The N-terminal amino acid sequence of this protein is set forth in the sequence listing herein as SEQ ID. NO: 1.

3. "Mammal" as used herein includes both humans and non-humans.

4. "mAb" refers to a monoclonal antibody.

5. "Substantially pure", as used to describe YKL-40, refers to the substantially intact molecule or fragments thereof which is essentially free of other molecules with which YKL-40 may be found in nature.

6. "Disease state" refers to an illness or injury in a mammal.

7. "Associated" with respect to the role in YKL-40 in a disease state in a mammal refers to releas of YKL-40 into a tissue or fluid of the mammal which release occurs during or at the onset of the disease state and is the result of the onset or occurrence of the disease state.

9. "Ameliorate" refers to a lessening in the severity of a disease state, including remission or cure thereof.

B. Isolation and Purification of YKL-40.

To develop antibodies for use in all assay procedures and antigen for use in competitive assay procedures according to the methods of the invention, YKL-40 must be obtained from a biological sample or synthesized, preferably in a substantially pure form. Native YKL-40 may be obtained from any mammalian fluid or tissue known to contain it. Although the normal distribution of YKL-40 in mammals is as yet not completely known, it has been found in serum, synovial fluid and conditioned media of chondrocytes and osteosarcoma cells (MG63 cell line, American Type Culture Collection, Rockville, MD. ["ATCC"]).

Preparation of conditioned media of cells according to means known in the art, preferably using RPMI 1640 serum-free media (Irvine Scientific, Irvine, CA.). YKL-40 is purified according to means known in the art, such as by affinity chromatography or gel filtration (on, for example, the resin SEPHACRYL S-200-HR from Pharmacia, Piscataway, N.J.).

YKL-40 has a molecular weight of about 40 kD. Its N-terminal amino acid sequence is shown in the sequence listing as SEQ ID. NO. 1. Substantial homology along the N-terminal and internal amino sequences (the latter of which are shown in SEQ ID NO. 2, ("YKL-40 peptide A") and SEQ. ID. NO. 3, ("YKL-40 peptide B")) with a bacterial polysaccharide hydrolase (chitinase) supports the conclusion that YKL-40 degrades polysaccharide components in connective tissue. Specifically, SEQ ID. NO. 2 correlates to 14/19 residues of an internal amino acid sequence for chitinase, while 50% of the residues in the N-terminal sequ nce for YKL-40 correlate to the N-terminus of chitinase (SEQ. ID. NO. 3).

YKL-40 may also be synthesized according to means which are well-known in the art. Using conventional techniques, the full-length sequence of the gene encoding for YKL-40 and/or the amino acid sequence thereof may be deduced by those of ordinary skill in the art using the information provided in any or all of SEQ. ID. NO. 1 through NO. 3. The full-length gene can be expressed using suitable expression vectors known in the art or the peptide can be chemically constructed using amino acids corresponding to the deduced amino acid sequence for YKL-40.

C. Antibodies to YKL-40.

Either polyclonal or monoclonal antibodies may be used in the immunoassays of the invention. Polyclonal antibodies may be raised by multiple subcutaneous or intramuscular injections of substantially pure YKL-40 into a suitable non-human mammal. The antibodies are then obtained from bleeds taken from the mammal. The techniques used to develop polyclonal antibodies are known in the art (see, e.g., Methods of Enzymology, "*Production of Antisera With Small doses of Immunogen: Multiple Intradermal Injections*", Langone, et al. eds. (Acad. Press, 1981)).

The general method used for production of hybridomas secreting monoclonal antibodies is well known (Kohler and Milstein, *Nature*, 256:495, 1975). The isolation of hybridomas secreting monoclonal antibodies reactive to YKL-40 can be accomplished using routine screening techniques to determine the elementary reaction pattern of the mAb of interest.

D. Immunoassay Procedures

The immunoassay procedure used must be quantitative so that levels of YKL-40 in a patient with disease may be distinguished from normal levels which may be present in healthy humans and/or background levels measured in the patient. Competitive assays on a solid phase using detectible labels (direct or indirect) are, therefore, preferred. The label will provide a detectible signal indicative of binding of antibody to the YKL-40 antigen. The antibody or antigen may be labelled with any label known in the art to provide a detectible signal, including radioisotopes, enzymes, fluorescent molecules, chemiluminescent molecules, bioluminescent molecules and colloidal gold. Of the known assay procedures, radioimmunoassay (RIA) is most preferred for its sensitivity. A radioisotope will, therefore, be the preferred label.

Examples of metallic ions which can be directly bound to an antibody, or indirectly bound to the YKL-40 antigen are well-known to those of ordinary skill in the art and include ^{125}I, ^{111}In, ^{97}Ru, ^{67}Ga, ^{68}Ga, ^{72}As, ^{89}Zr, ^{90}Y and ^{201}Tl. Preferred for its ease of attachment without compromise of antigen binding specificity is ^{125}I (sodium salt, Amersham, United Kingdom). Labelling of YKL-40 with ^{125}I may be performed according to the method described in Salacinski, *et al., Anal. Biochem.*, 117:136-146, 1981. Iodogen for use to provide the ^{125}I label (1,3,4,6-tetrachloro-3α, 6α-diphenyl glycoluril) is commercially available from Pierce and Warriner, Chester, England.

The radioimmunoassay of the invention uses standards or samples incubated with a substantially equal volume of YKL-40 antiserum and of YKL-40 tracer. Standards and samples are gen rally assayed in duplicate. The s nsitivity (detection limit) of the assay of the invention is about 10μg/L. Sensitivity in this context is defined as the detectible mass equivalent to twic the standard deviation of the zero binding values. The standard curve will generally be

linear between 20 and 100 μg/L. The intra- and interassay coefficients of variance for the assay described in the following examples are <6.5% and <12%, respectively.

It will be appreciated by those skilled in the art that, although not as sensitive as an RIA, assay procedures using labels other than radioisotopes have certain advantages and may, therefore, be employed as alternatives to the preferred RIA format. For example, an enzyme-linked immunosorbent assay (ELISA) may be readily automated using an ELISA microtiter plate reader and reagents which are readily available in many research and clinical laboratories. Fluorescent, chemiluminescent and bioluminescent labels have the advantage of being visually detectible, though they are not as useful as radioisotopes to quantify the amount of antigen bound by antibody in the assay.

E. Diagnostic Application

As shown in examples provided below, diagnosis of disease based on measured levels of YKL-40 can be made by comparison to levels measured in a disease-free control group or background levels measured in a particular patient. The diagnosis can be confirmed by correlation of the assay results with other signs of disease known to those skilled in the clinical arts, such as the diagnostic standards for RA and breast cancer described in the examples below.

Where the amelioration of a disease state (such as RA) can be related to reduction in levels of YKL-40 (and concomitant cartilage repair), YKL-40 levels in a biological assay sample taken from the patient (e.g., synovial fluid) should be measured befor (for background) and periodically during the course of treatment. Because reductions in YKL-40 levels may be transient, the assay will preferably be performed at regular intervals (e.g., very 4 weeks) closely

before and after each treatment. Depending on the course of treatment, tumor load and other clinical variables, clinicians of ordinary skill in the art will be able to determine an appropriate schedule for performing the assay for diagnostic or disease/treatment monitoring purposes.

Because in certain instances serum YKL-40 may stem from sources other than the tissue of interest, a sample should, if possible, be taken from the tissue of interest. For example, for diagnosis or monitoring of joint disease the assay sample will preferably be drawn from the synovial fluid of the affected or potentially affected joint. For diagnosis and monitoring of tumor metastasis, however, the preferred source for the assay sample will be blood. Those of ordinary skill in the art will be able to readily determine which assay sample source is most appropriate for use in diagnosis of a particular disease for which YKL-40 is a marker.

The levels of YKL-40 which are indicative of the development or amelioration of a particular disease will vary by disease and, to a lesser extent, by patient. Generally, however, for purposes of diagnosing the onset or amelioration of disease, variations in the levels of YKL-40 of interest will be those which are statistically significant and which correlate to other clinical signs of disease occurrence and/or amelioration known to those skilled in the clinical art pertaining to the disease of interest.

For example, in rheumatic joint diseases synovial fluid YKL-40 levels can be correlated to other biochemical markers of joint disease, in particular elastolytic activity by monocytes and macrophages for the degradation of proteoglycans and collagens in synovial fluid. YKL-40 levels also correlate well to elevated IL-6 levels in synovial fluid. IL-6 is secreted by chondrocytes and synovial cells and serves to r gulate immun responses, including inflammation. Relatively

high levels of IL-6 are found in the synovial fluid of patients with inflammatory and degenerative arthropathies.

Correlation also exists to a somewhat lesser extent between YKL-40 levels and acute C-reactive protein (CRP) levels. CRP is present in elevated quantities in the acute phase of rheumatic joint diseases and appears to play a biologic role in inflammation. YKL-40 levels similarly correlate with serum P-III-NP levels, which reflect local inflammatory alterations in type III collagen metabolism in synovial fluid. Although it is not intended that the invention be limited to a particular diagnosis, the correlation of YKL-40 levels suggests that its levels may in particular be indicative of inflammation in joint disease.

By way of further example, in breast cancer patients, serum YKL-40 levels are elevated in patients with cancer cell metastasis as compared to patients without breast cancer. It is probable that the elevated levels of YKL-40 in serum are produced at least in part by degeneration of the connective barrier to the entrance of cancer cells into blood. It can be expected that a similar process may accompany entrance of cancer cells into lymphatic circulation.

Interestingly, greatly elevated levels of YKL-40 appear in patients who have experienced a recurrence of breast cancer. Moreover, elevation of serum levels of YKL-40 correlate to the number of months each patient can be expected to survive following recurrence of the cancer. Generally, the higher the level of YKL-40, the shorter the period of survival.

Examples illustrating the correlation of YKL-40 levels to joint disease activity, progress of treatment for joint disease, canc r cell metastasis and cancer survival rates are provided below.

EXAMPLE I

ISOLATION AND PURIFICATION OF YKL-40 FROM HUMAN OSTEOSARCOMA CELL LINE MG63

YKL-40 was purified from serum-free conditioned medium of the human osteosarcoma cell line MG63 (MG63 cells were obtained from the American Type Culture Collection, Rockville, Maryland). Cells were cultured in 100 mm dishes with RPMI 1640 medium containing 10% newborn calf serum, 100 Units/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml vitamin C, and 1 μg/ml vitamin K_1. The cultures were incubated at 37°C in a humidified atmosphere of 10% CO_2. When the cells reached confluence, the culture medium was removed and the cell layer was washed twice with 10 milliliters (ml) of phosphate buffered saline.

Ten mls of serum-free RPMI 1640 media containing 50μg/ml vitamin C and 1 μg/ml vitamin K_1 was then added to each dish. 48 hours later, conditioned medium was decanted from each dish and replaced with 10 ml of fresh serum-free medium containing the same level of added constituents. This procedure was repeated every 48 hours for up to 10 days. Conditioned medium was freed of cells and debris by centrifugation and stored at -20°C until use.

YKL-40 was purified by a modification of the heparin-affinity chromatography method described in Nyirkos, *et al., Biochem. J.*, 268:265-268, 1990. Specifically, YKL-40 was first concentrated from 4.75 L of conditioned medium by adsorption of 40 ml (packed volume) of HEPARIN-SEPHAROSE CL-6B resin (from Pharmacia) by stirring for 2 hours at room temperature. The resin was then placed into a 2 x 24 cm column and washed with 3 column volumes of 0.01 Molar sodium phosphate buffer (pH 7.4) containing 0.05 M NaCl. YKL-40

was eluted from the resin at room temperature by a linear gradient from 0.05 to 1.2 M NaCl in 0.01 Molar sodium phosphate buffer pH 7.4 (200 ml each condition).

To characterize the purity of YKL-40, 5 μl from every third fraction of the peak fractions from the HEPARIN-SEPHAROSE CL-6B affinity chromatography procedure described were combined with 25 μl SDS loading buffer electrophoresed on a 5-20% SDS-polyacrylamide gradient gel (BioRad, Laboratories, Richmond, CA), and stained with Coomassie brilliant blue. The concentration of the final YKL-40 used for standard and tracer in the inventive assay is based on an absorbance of 1.44 for a 1 milligram (mg) per ml solution of YKL-40.

Articular cartilage was obtained from the knees of cadavers within 18 hours of death and of a patient undergoing joint replacement for osteoarthritis, and chondrocytes were isolated by sequential enzymatic digestion according to methods known in the art (see, e.g., Guerne, *et al.*, *J. Immun.*, 144:499-505, 1990). The resulting cells were a homogenous population of chondrocytes, since only the superficial layers of cartilage were used for isolation of the cells and, in contrast to fibroblasts or synoviocytes, the cells were nonadherent.

The cells were cultured in DMEM-high glucose medium supplemented with 10% fetal calf serum, 100 Units/ml of penicillin, 100μg/ml streptomycin, and 50 μg/ml vitamin C (Irvine Scientific, Irvine, California). Cells were grown in 175 cm^2 tissue culture flasks (primary cultures) or in 100 mm dishes (later passages) in a humidified atmosphere of 10% CO_2 at 37°C. The cells were subcultured at a 1:3 ratio after trypsinization of confluent monolayers. To obtain conditioned medium for analysis, the culture medium was r mov d after the cells reached confluence and the cell lay r was washed twice with 30 ml (175 cm^2 flasks) or 10 ml (100 mm dishes) of phosphate buffered saline (PBS). The same volume of serum-fr e DMEM-high glucos medium containing antibiotics, and 50 μg/ml

vitamin C was then added to each culture. Conditioned medium was removed after 48 hours and replaced with the same volume of fresh serum-free medium. This procedure was repeated every 48 hours for up to 14 days. Conditioned medium was freed of cells and debris by centrifugation for 5 minutes at 1600 g and frozen at -20°C until use.

EXAMPLE II

PREPARATION OF ASSAY SAMPLES FOR RADIOIMMUNOASSAY

Assay Sample Sources

Assay samples were obtained from the sera of 49 patients with inflammatory or degenerative joint diseases (34 women and 15 men, aged 23-80 years with a median age of 65 years). 29 patients had RA, 7 had osteoarthritis, 4 had crystal arthritis, 2 had psoriatic arthritis, 5 had reactive arthritis and 2 had monarthritis. Diagnoses were based on the criteria described in Arnett, *et al.* (1988) *Arthritis Rheum.* 31: 315-324 (American Rheumatism Association Standards), clinical and radiographic examinations of the knees, and direct microscopy of synovial fluid. The patients had a serum CRP level of 25-1600 (median 165). 34 patients were taking non-steroidal anti-inflammatory drugs and 17 were receiving slow acting antirheumatic agents. 15 patients had received glucocorticoid therapy systemically or locally within the past 3 months. The inflammation of the knee was evaluated by a clinical index rating from 0-6, consisting of palpable synovial swelling (range 0-3) and pain on palpation (0-3).

Collection of Serum and Synovial Fluid

Blood samples w re allowed to clot at room temperature and then centrifuged at 1500 g for 10 minutes. Knee joint aspirations were performed using conventional aseptic technique without local anesthesia. The synovial fluid was withdrawn from each subject as completely as possible using a 1.2-mm-gauge

needle, and collected in sterile tubes containing ethylene-diamine-tetracetate (EDTA, 5 mM final concentration). The synovial fluid samples were centrifuged at 1800 g for 30 minutes in order to remove any extraneous debris. The samples were either analyzed immediately or stored at -80°C for later analysis.

EXAMPLE III

PREPARATION OF LABELLED ANTIGEN AND ANTIBODIES FOR RADIOIMMUNOASSAY FOR YKL-40

Preparation of Radioiodinated YKL-40

Purified YKL-40 was labelled with ^{125}I (sodium salt, Amersham, UK) according to the Iodogen method referenced supra. Specifically, 10 μg YKL-40 was incubated for 10 minutes with 18.5 MBq ^{125}I using 2 μg of iodogen (Pierce and Warriner, Chester, England, UK) as oxidant in a reaction volume of 110 μl. Iodination was terminated by moving the reaction mixture from the iodogen tube. The labelled YKL-40 was separated from free iodine by gel filtration using a SEPHADEX G-25 column (1 x 12.5 cm, from Pharmacia) equilibrated with assay buffer (16 mM sodium phosphate buffer pH 7.4, 0.12 M NaCl, 0.1% (w/v) human serum albumin). The calculated specific activity of the labelled was about 15 Ci/g. The elution position of YKL-40 (purified) and of YKL-40 taken from the serum of a patient with RA is shown in Figure 1.

Preparation of Antibodies

New Zealand white rabbits were immunized by monthly multiple site subcutaneous or intramuscular injection of purified YKL-40. Each injection was made with 0.5 mg of human YKL-40 emulsified in incomplete Freund's adjuvant (1:1). The first 4 injections were given at intervals of two w eks and rabbits wer bled 10-12 days after th fourth injection. Injections wer thereafter given at 4 week

intervals and the animals were bled 10-12 days after each injection. Crossed immunoelectrophoresis showed that the antibodies were monospecific for YKL-40.

It will be understood by those skilled in the art that the radioisotopic label could be attached to the antibodies described above rather than the antigen with functional equivalence in the assay claimed.

EXAMPLE IV
RADIOIMMUNOASSAY FOR YKL-40

The assay samples described in Example II were assayed as follows. YKL-40 antibodies, standards and the tracer were diluted in assay buffer. In the assay 100 μl of standards or samples were incubated with 100 μl of YKL-40 antiserum (1:10,000) and 100μl of YKL-40 tracer (about 15,000 counts/minute) in a final volume of 400 μl at room temperature for 20-24 hours. The antibody-bound tracer was then separated by incubation with 100 μl of SAC-CEL (donkey anti-rabbit antibody coated cellulose suspension; Wellcome Diagnostics Ltd, UK) at room temperature for 30 minutes. After addition of 1 ml of distilled water the tubes were centrifuged at 2000 g for 10 minutes, the supernatant decanted, and the radioactivity of the precipitate counted in an automatic gamma counter (LKB Wallac, CLINIGAMMA 1272) for the time of 10,000 counts.

The precision (intra-assay variation) was calculated from replicate determinations (20 times) on each of three quality control sera in a single assay. The reproductibility (inter-assay variation) was calculated from data obtained during a 5 month period (20 assays) on each of three quality control sera. YKL-40 concentrations in c rresponding serum and EDTA plasma samples were compared in 75 blood donors.

A typical standard curve for this RIA is shown in Figure 2. All standards and samples were assayed in duplicate. The standard curve was constructed by use of a spleen function.

The individual serum YKL-40 concentrations in the two patient groups and controls are shown in Figure 2. The serum YKL-40 concentrations of patients with inflammatory rheumatic disease (median; lower quartile-upper quartile: 138 μg/L; 103-211 μg/L) was not statistically different (p=0.44) from those in patients with osteoarthritis (112 μg/L; 93-152 μg/L). Serum YKL-40 in both patient groups was significantly higher (p<0.001) than that of controls (50 μg/L; 36-64 μg/L). The YKL-40 concentration in knee joint synovial fluid from the patients with inflammatory rheumatic disease (2210 μg/L; 1625-3040 μg/L) was not significantly different from the concentration of the patients with osteoarthritis (1720 μg/L; 1270-1950 μg/L).

Serum levels of YKL-40 can, therefore be related to the incidence of joint disease, particularly inflammatory joint disease. However, distinctions between the different joint diseases evaluated are not apparent from this assay.

EXAMPLE V

YKL-40 STABILITY IN SERUM ASSAY SAMPLES

To assess the effect of freezing and thawing on YKL-40 antigen in the assay samples, a fresh serum sample was obtained from 6 adults and 10 aliquots of each sample were prepared. One aliquot was kept on ice, and the others were frozen at -20°C. At 60 minute intervals, the aliquots were removed and thawed at room temperature. One sample was kept on ice and the rest refrozen. This procedure was repeated 9 times with no loss of serum YKL-40 reactivity. To assess the effect of long-term storage at room temperature, a fresh serum sample was obtained from 12 adults, and 4 aliquots of each

sample were prepared. One aliquot was immediately frozen at -20°C, the others were frozen after 24 hours, 48 hours and 120 hours storage at room temperature, during which time reactivity remained stable.

EXAMPLE VI

CORRELATION BETWEEN YKL-40 IN SERUM AND SYNOVIAL FLUID AND OTHER BIOCHEMICAL MARKERS OF INFLAMMATION AND CARTILAGE REMODELING

As shown in FIGURE 3, the YKL-40 concentration measured in the assay samples described in Example II and the synovial fluid/serum YKL-40 ratio was high; i.e., highly correlated (15.0; 11.8-19.1). YKL-40 levels in serum and synovial fluid correlated significantly with serum CRP, synovial fluid IL-6, and synovial fluid Mϕ elastolysis levels, which were also measured by assay of these samples as described below. Serum YKL-40 also correlated with serum Mϕ elastolysis and serum P-III-NP levels in these samples. The synovial fluid YKL-40 concentrations measured in these samples correlated with a clinical index of knee inflammation. No correlation was found between YKL-40 in serum or synovial fluid and serum IL-6 and synovial fluid P-III-NP levels.

The serum concentration of CRP was determined by nephelometry (Behringwerke, Marburg, Germany). Interleukin-6 (IL-6) activity was determined by bioassay using the highly specific IL-6 dependant mouse hybridoma cell line B13, 29 clone B9 known in the art. The aminoterminal propeptide of type III procollagen (P-III-NP) was measured by a commercially available RIA (P-III-NP RIA kit, Farmos Diagnostica, Oulunsalo, Finland). The elastolytic activity of monocytes/macrophages (Mϕ) wer investigated with an assay for live Mϕ elastolysis described by Jensen, et al. (1991) *Scand.J.Rheum.* 20:83-90. The results of thes assays are shown in Table I, below.

TABLE I

RELATIONSHIP BETWEEN SERUM AND SYNOVIAL FLUID CONCENTRATIONS OF YKL-40 AND OTHER BIOCHEMICAL MARKERS OF JOINT DISEASES

	Serum YKL-40	Synovial fluid YKL-40
Serum CRP	0.33*	0.31*
Serum IL-6	0.26	-0.10
Synovial fluid IL-6	0.60**	0.47*
Serum Mϕ elastolysis	0.55**	0.58**
Synovial fluid Mϕ elastolysis	0.64**	0.58**
Serum PIIINP	0.49***	0.13
Synovial fluid PIIINP	0.02	-0.23
Clinical Knee Index	0.27	0.34*

Correlations are given as Spearman's rho/p values. *p<0.05; **p<0.01; ***p<0.001.

EXAMPLE VII

CORRELATION OF CHANGES IN SERUM YKL-40 LEVELS WITH PROGRESS IN THE TREATMENT OF JOINT DISEASE

The study included 97 patients with definite or classic rheumatoid arthritis (RA) as defined by the American Rheumatism Association (see Arnell, *et al.*, Arthritis Rheum., supra). The patients entered a double blind placebo controlled trial of pulse treatment with 1000 mg intravenously injected methylprednisolone (MP) very 4 weeks for a total of six (6) times, followed by six months without

pulse therapy. 7 days after the first pulse therapy the patients were started on penicillamine or azathioprine as described in Hansen, *et al.* (1990) Br. Med. J. 301:268. 57 patients completed the trial, taking the same disease modifying drug throughout (31 were treated with MP and 26 with placebo).

Blood samples were collected in the morning just before each pulse treatment and plasma YKL-40 was determined by RIA as described in Example V. The initial level of plasma YKL-40 was 174 μg/L (median) (108-261 μg/L) (median) (lower quartile-upper quartile) in the 97 patients with RA and significantly higher ($p<0.001$) than was observed in 275 healthy adults (104 μg/L (83-143 μg/L).

In the MP treated group a significant decrease ($p<0.01$) was found in YKL-40 24 hours after start of treatment. Furthermore, plasma YKL-40, measured after 4, 8, 12, 16, and 20 weeks of treatment with MP, was significantly lower (p<0 0.05 - p<0.001) compared to the initial values. Six months after withdrawal of MP therapy plasma YKL-40 had returned to baseline values (FIGURE 4, O).

In the placebo treated group, plasma YKL-40 was significantly lower (p<00.05) compared to the initial values at 12 weeks (FIGURE 4, •).

The effects of MP treatment on YKL-40 levels were also compared to its effect on other biochemical markers of joint disease. Serum hyaluronan was determined by a radiometric assay using specific hyaluronan-binding protein isolated from bovine cartilage (Pharmacia, Uppsala, Sweden). Serum C reactive protein (CRP) was determined by nephelometry (Beringwerke, Marburg, Germany).

The changes in plasma YKL-40 during MP treatment was different compared to the changes in serum CRP and serum hyaluronan, as shown in FIGURE 5(a) and (b). Correlation between measured changes in the levels of YKL-40 and

CRP/hyaluronan markers was significant at the start of treatment ($r=0.52$, $p<0.001$ for CRP; $r=0.41$, $p<0.001$ for serum hyaluronan; $r=0.37$, $p<0.001$ for the number of swollen joints), and declined in correlation at the end of 12 months.

MP therapy therefore produced a significant by transient decrease in plasma YKL-40 which corresponded in the first months following treatment to other indicators of a therapeutic response.

EXAMPLE VIII

RELATIONSHIP OF SERUM YKL-40 LEVELS TO SURVIVAL RATES FOLLOWING RECURRENCE OF BREAST CANCER

Serum levels of YKL-40 were measured in a clinical group of 60 breast cancer patients (aged 29-78 years) using the RIA described in Example IV. For comparison, serum YKL-40 levels in a control group of 137 disease-free women (aged 20-79 years) were also measured. These latter measurements define the normal and median YKL-40 values referred to in this example.

The following definitions apply to the following discussion of the study and results obtained from it.

1. "Normal" YKL-40 levels refers to serum concentrations (as measured in the control group) of 164 μg/L or less.

2. "Median Range" of YKL-40 levels refers to serum concentrations of 80-164 μg/L (as measured in the control group. These values correspond to the 20-80% percentile range of measured serum levels in the control group. The median value in this group is 104 μg/L.

3. "Aberrant" YKL-40 levels refers to concentrations of YKL-40 measured in the clinical group which were in excess of any serum level measured in control group members of age 70 or younger.

4. "Specificity" refers to the proportion of clinical group members who did not have metastasis to bone and received "negative" test results for bone metastasis.

5. "Sensitivity" refers to the proportion of clinical group members who had metastasis to bone and received "positive" test results for bone metastasis.

The statistical analyses were done using commercially available statistical software. Results are expressed as median, range or percentiles. Patient survivals were analyzed by use of the Kaplain-Meier method and the significant of differences in survival curves were determined with the log-rank method known in the art of statistical analysis.

The members of the clinical and control groups were, respectively:

<u>Clinical Group</u>:

60 women aged 29-78 years who had previously been diagnosed with primary breast cancer. They were all potential candidates for systemic antineoplastic treatment. The criteria of entry were: 1) Suspicion of distant metastases after primary treatment of localized disease; 2) Locally advanced disease or distant metastases at the time of initial diagnosis; and 3) Patients with suspected progression of bone metastasis after initial recurrence. Patients who had other primary cancers at any time wer not eligible for this study.

39 patients (65%) had received adjuvant therapy. 22 (56%) of these patients had received adjuvant combination chemotherapy with cyclophosphamide, methotrexate and 5-fluorouracil immediately after the removal of the primary tumor. None of the patients had been treated during the previous 12 weeks before the start of the study (i.e., the time of assay sample collection).

Control Group

Serum YKL-40 concentrations in 137 healthy women (aged 20-79 years) were established for use as control values. The serum samples were obtained from blood donors who attended the Regional Blood Transfusion Services at Hvidovre Hospital, Denmark, from women working at different museums in Copenhagen, Denmark and from elderly women living in a shared house for elderly in Copenhagen. All these women were healthy (had no known disease), were not taking any medicine and all had a normal liver and kidney function.

The period of time which each patient in the clinical group survived following recurrence of their cancer was observed. The nature of any metastasis of the tumor cells was also characterized in each patient. These data are correlated to the serum YKL-40 levels measured in each patient at the time of recurrence of their cancer.

FIGURE 6 shows the Kaplain-Meier survival curve analysis is relating serum levels of YKL-40 measured in blood samples taken from each member of the clinical group at the time that the first recurrence of their cancer was observed (as indicated by metastasis of tumor cells from breast tissue) to length of survival of each patient (in months) following recurrence. Of the women who had serum levels of YKL-40 abov the 80th percentile (i.e., abov th high normal ceiling of 164 μg/L), 61% died within 12 months. In contrast, 76% of patients with serum levels of YKL-40 below 164 μg/L were still alive after 16

months. By 24 months, 82% of the patients with higher than normal serum YKL-40 levels had died, while only 47% of the "normal" patients in the clinical group had died.

Similar Kaplain-Meier curves based on serum levels of other blood proteins (such as serum alkaline phosphatase) measured at the same time as the YKL-40 levels did not correlate as clearly to survival rate among the clinical group embers (see, Table II below).

TABLE II

THE DIAGNOSTIC VALUES OF SERUM YKL-40, SERUM BGP AND SERUM ALKALINE PHOSPHATASE IN THE DIAGNOSIS OF BONE METASTASES

	YKL-40	BGP	Alkaline Phosphatase
Key diagnosis: X-ray examination			
Sensitivity	55% (60%)*	30%	45%
Specificity	61% (86%)	78%	83%
PV (Pos)	67% (93%)	67%	80%
PV(Neg)	48% (41%)	44%	53%
Key diagnosis: Bone marrow biopsy			
Sensitivity	64%	39%	56%
Specificity	68%	82%	91%
PV(Pos)	70%	69%	88%
PV(Neg)	63%	56%	65%

*In parentheses is shown the values if the bone status was evaluated independent of the test results.

There was no clear relationship between the level of serum YKL-40 and the anatomical location of metastases or other clinical parameters, such as the menopausal status of each patient (see Table III, below). However, serum YKL-40 values were elevated compared to normal levels in 65% of the patients with visceral metastasis compared to 46% of the patients with soft tissue metastasis.

There also did not appear to be any clear relationship between YKL-40 levels and age, although, as shown in FIGURE 7, aberrant levels of YKL-40 did not appear in healthy (control group) women below age 70.

TABLE III

SERUM YKL-40 IN RELATION TO DIFFERENT CLINICAL PARAMETERS IN 60 WOMEN WITH FIRST RECURRENCE OF BREAST CANCER

	Serum YKL-40 μg/L
Menopausal status	
Premenopausal (N=30)	141 (39-1170)
Postmenopausal (N=29)	157 (61-1152)
Anatomical location of metastases	
Soft Tissue (n=28)	195 (61-1170)
Bone (n=33)*	179 (11-1170)
Visc ra (n=17)	233 (39-1170)

Values are expressed as median (range).

*Determined by X-ray

As shown in FIGURE 8, 76% of the clinical group members still alive after 16 months following recurrence had serum YKL-40 levels of 164 μg/L or less. 85% of the members who lived longer than 30 months following recurrence had serum YKL-40 levels of 164 μg/L or less.

Relating serum YKL-40 levels to the presence or absence of one or more bone metastases, YKL-40 levels were elevated in clinical group members with positive test results as opposed to negative test results. In addition, YKL-40 levels were elevated in positive test result members with more than one metastasis to bone as opposed to members with one metastasis to bone. As compared to other blood proteins measured (see Table II), YKL-40 levels may, therefore, have diagnostic value with respect to metastases of breast cancer cancer cells to bone.

The individual YKL-40 serum levels on which the percentages in Table I are based are shown in FIGURE 9 (levels in women diagnosed with bone metastasis based on X-ray evaluations) and in FIGURE 10 (YKL-40 levels measured in women diagnosed with bone metastasis based on histological evaluations of bone marrow biopsies).

The invention being fully described, it will be apparent to those of skill in the art that modifications may be made to the embodiments described above without departing from the spirit or scope of the invention.

SUMMARY OF SEQUENCES

Sequence ID NO. 1 is the N-terminal amino acid sequence for the YKL-40 protein.

Sequence ID NO. 2 is an internal amino acid sequence for the YKL-40 protein ("YKL-40 Peptide A" in the application).

Sequence ID NO. 3 is another internal amino acid sequence for the YKL-40 protein ("YKL-40 Peptide B" in the application).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: PRICE, PAUL A.
JOHANSEN, JULIA S.

(ii) TITLE OF INVENTION: ASSAY FOR YKL-40 AS A MARKER FOR DEGRADATION OF MAMMALIAN CONNECTIVE TISSUE MATRICES

(iii) NUMBER OF SEQUENCES: 3

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: SPENSLEY HORN JUBAS & LUBITZ
(B) STREET: 1880 CENTURY PARK EAST, FIFTH FLOOR
(C) CITY: LOS ANGELES
(D) STATE: CALIFORNIA
(E) COUNTRY: USA
(F) ZIP: 90067

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE: 09-JUL-1993
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: HOWELLS, STACY L.
(B) REGISTRATION NUMBER: 34,842
(C) REFERENCE/DOCKET NUMBER: PD2759

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 619/455-5100
(B) TELEFAX: 619/455-5110

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(B) CLONE: YKL-40 N-TERMINAL SEQUENCE

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
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1               5                   10                  15

Asp Gly Ser Xaa Phe Pro Asp Ala Leu
            20                  25
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(B) CLONE: YKL-40 INTERNAL PEPTIDE A

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..19

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Leu Asn Thr Leu Lys Asn Arg Asn Pro Asn Leu Lys Thr Leu Leu Ser
1               5                   10                  15

Val Gly Gly
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(B) CLONE: YKL-40 INTERNAL PEPTIDE B

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..7

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Leu Arg Leu Gly Ala Pro Ala
1               5
```

CLAIMS:

1. A method for identifying the presence of a disease state in a mammal which is associated with degradation of connective tissue containing YKL-40, which method comprises detecting and quantifying the concentration of YKL-40 in a biological sample taken from the mammal.

2. A method according to Claim 1 wherein the method for detecting and quantifying YKL-40 comprises a competitive immunoassay which measures specific binding of substantially pure YKL-40 and any YKL-40 in the biological sample by an antibody, which binding is indicated by a signal provided by a detectible label attached to the antibody or to its corresponding YKL-40 antigen.

3. A method according to Claim 2 wherein the detectible label is selected from the group consisting of radioisotopes, enzymes, fluorescent molecules, chemiluminescent molecules, bioluminescent molecules and colloidal metals.

4. A method according to Claim 1 wherein the disease state is inflammatory or degenerative joint disease.

5. A method according to Claim 1 wherein the disease state is metastasis of tumor cells into the vascular system of the mammal.

6. A method according to Claim 5 wherein the disease state is metastatic breast cancer.

7. A method according to Claim 1 wherein the mammal is a human.

8. A method according to Claim 2 wherein the antibody is a polyclonal antibody which is specifically reactive to YKL-40.

9. A method according to Claim 2 wherein the antibody is a monoclonal antibody which is specifically reactive to YKL-40.

10. A method according to Claim 1 wherein the biological sample is a fluid or tissue sample taken from tissue affected by the disease state.

11. A method according to Claim 10 wherein the biological sample is blood or plasma.

12. A method for acquiring data which is suggestive of the prognosis for the length of survival of a breast cancer patient whose cancer has recurred following remission comprising detecting and quantifying the levels of YKL-40 in serum taken from the patient following recurrence of the cancer.

13. A method according to Claim 12 wherein the method for detecting and quantifying YKL-40 comprises a competitive immunoassay which measures specific binding of substantially pure YKL-40 and any YKL-40 in the biological sample by an antibody, which binding is indicated by a signal provided by a detectible label attached to the antibody or to its corresponding YKL-40 antigen.

14. A method according to Claim 13 wherein the detectible label is selected from the group consisting of radioisotopes, enzymes, fluorescent molecules, chemiluminescent molecules, bioluminescent molecules and colloidal metals.

15. A method according to Claim 13 wherein the antibody is a polyclonal antibody which is specifically reactive to YKL-40.

16. A method according to Claim 13 wherein the antibody is a monoclonal antibody which is specifically reactive to YKL-40.

17. A method for monitoring the progress or amelioration of a disease state in a mammal which is associated with degradation of connective tissue containing YKL-40, which method comprises detecting and quantifying the concentration of YKL-40 in a biological sample taken from the mammal.

18. A method according to Claim 17 wherein the method for detecting and quantifying YKL-40 comprises a competitive immunoassay which measures specific binding of substantially pure YKL-40 and any YKL-40 in the biological sample by an antibody, which binding is indicated by a signal provided by a detectible label attached to the antibody or to its corresponding YKL-40 antigen.

19. A method according to Claim 18 wherein the detectible label is selected from the group consisting of radioisotopes, enzymes, fluorescent molecules, chemiluminescent molecules, bioluminescent molecules and colloidal metals.

20. A method according to Claim 17 wherein the disease state is inflammatory or degenerative joint disease.

21. A method according to Claim 17 wherein th mammal is a human.

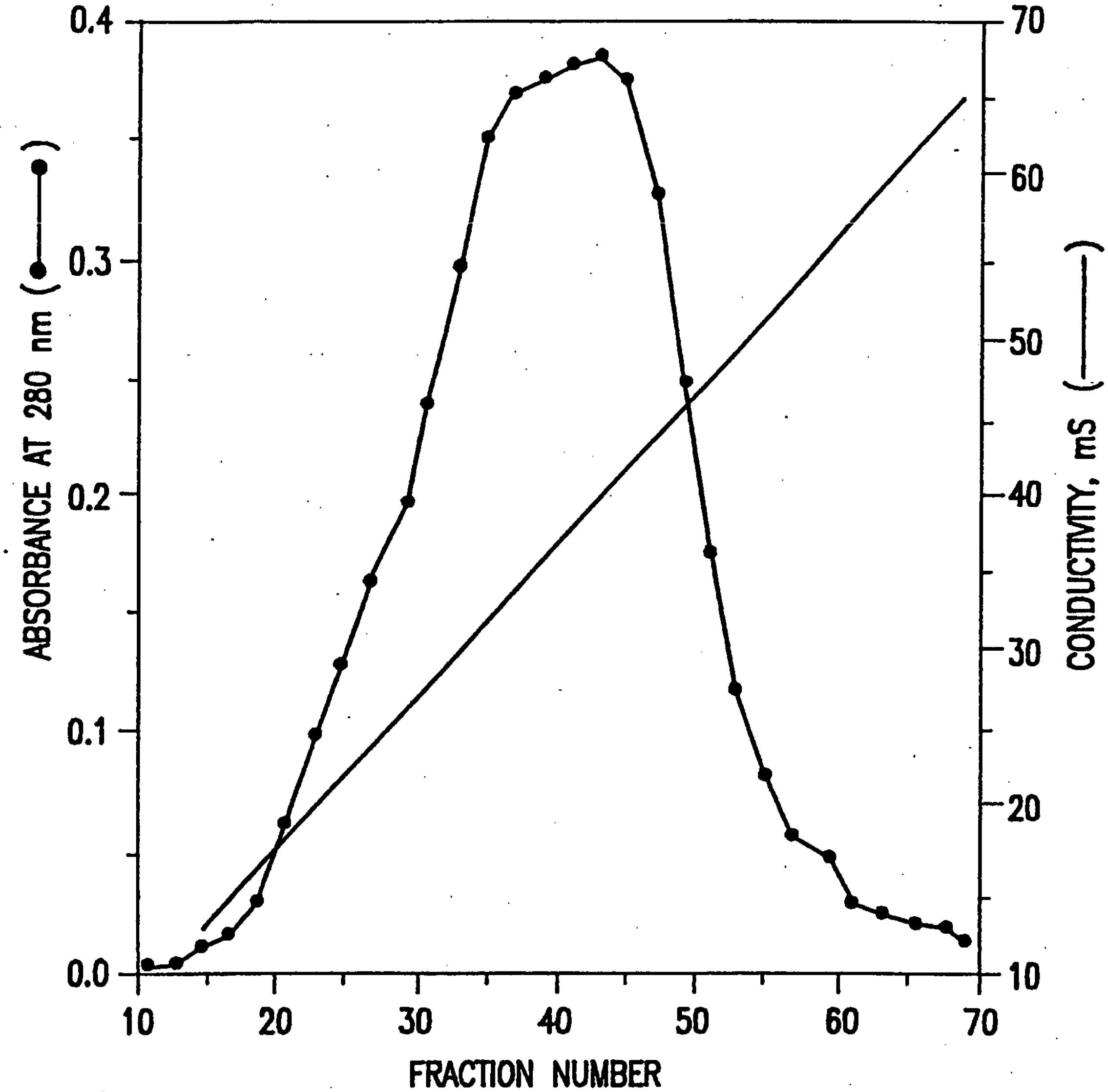
22. A method according to Claim 17 wherein the antibody is a polyclonal antibody which is specifically reactive to YKL-40.

23. A method according to Claim 17 wherein the antibody is a monoclonal antibody which is specifically reactive to YKL-40.

24. A method according to Claim 17 wherein the biological sample is a fluid or tissue sample taken from tissue affected by the disease state.

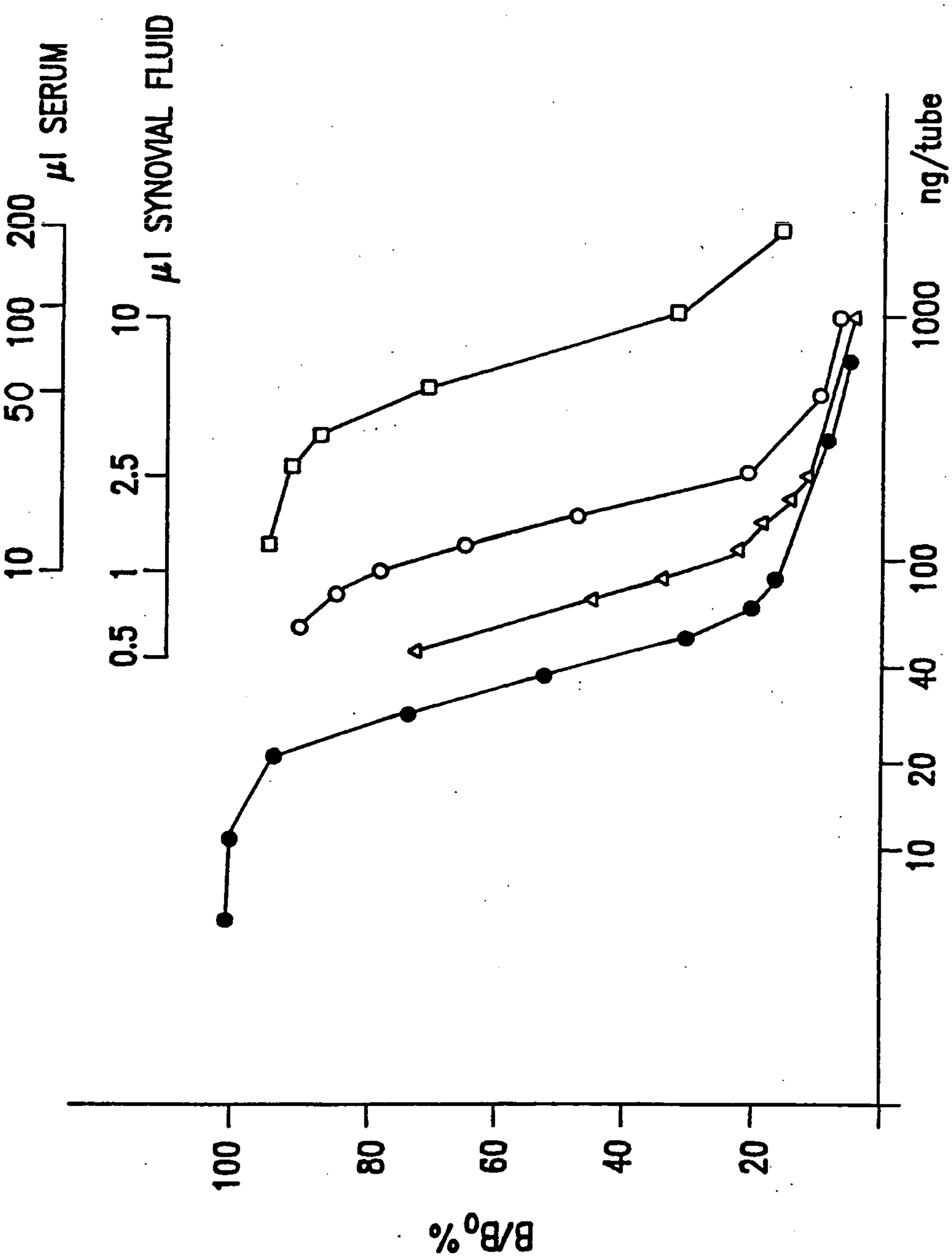
25. Polyclonal antibodies produced by immunization of a non-human mammal which antibodies are specifically reactive to YKL-40.

26. Monoclonal antibodies produced from hybridomas formed from cells taken from a non-human mammal which antibodies are specifically reactive to YKL-40.



0.4
0.3
0.2
0.1
0.0
ABSORBANCE AT 280 nm
10
20
30
40
50
60
70
FRACTION NUMBER
70
60
50
40
30
20
10
CONDUCTIVITY, mS

FIG.1



μl SERUM
10
50
100
200
μl SYNOVIAL FLUID
0.5
1
2.5
10
ng/tube
10
20
40
100
1000
B/B0%
100
80
60
40
20

FIG.2

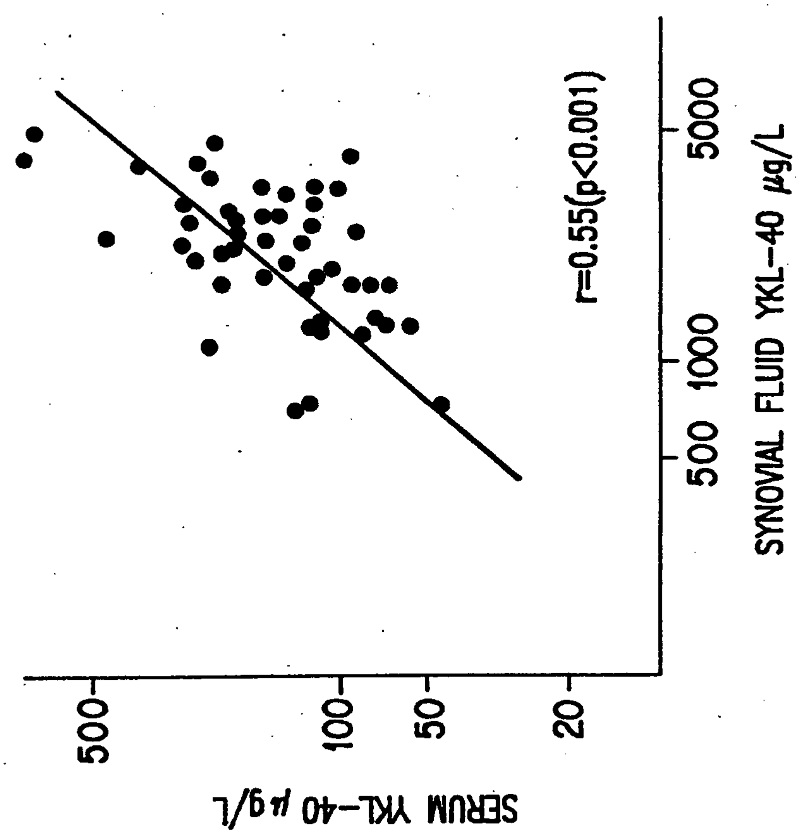
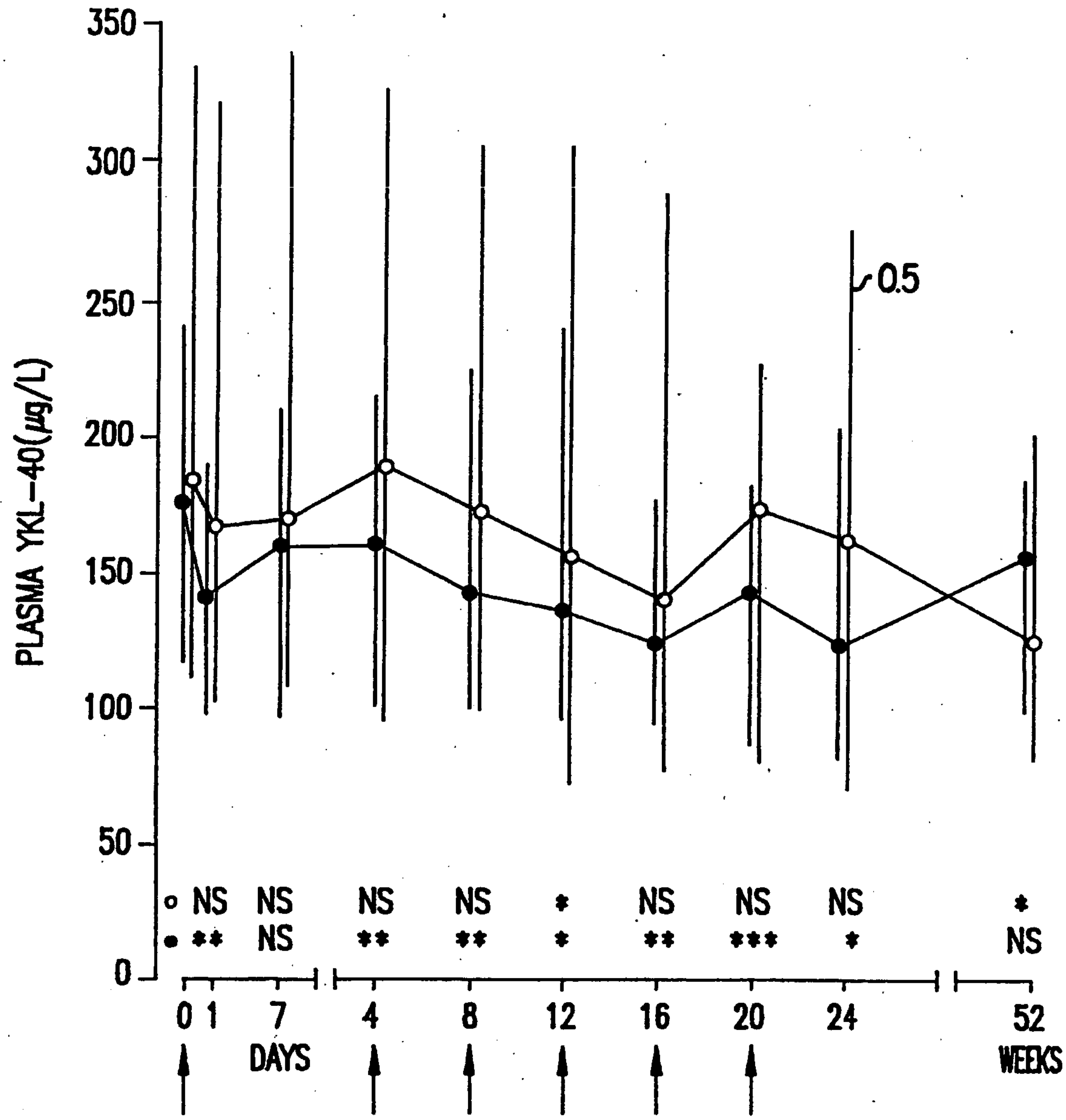

r=0.55(p<0.001)
500
100
50
20
SERUM YKL-40 µg/L
500
1000
5000
SYNOVIAL FLUID YKL-40 µg/L

FIG.3



350
300
250
200
150
100
50
0
PLASMA YKL–40(μg/L)
0.5
○ NS NS NS NS * NS NS NS *
● ** NS ** ** * ** *** * NS
0 1 7
DAYS
4 8 12 16 20 24
52
WEEKS

FIG.4

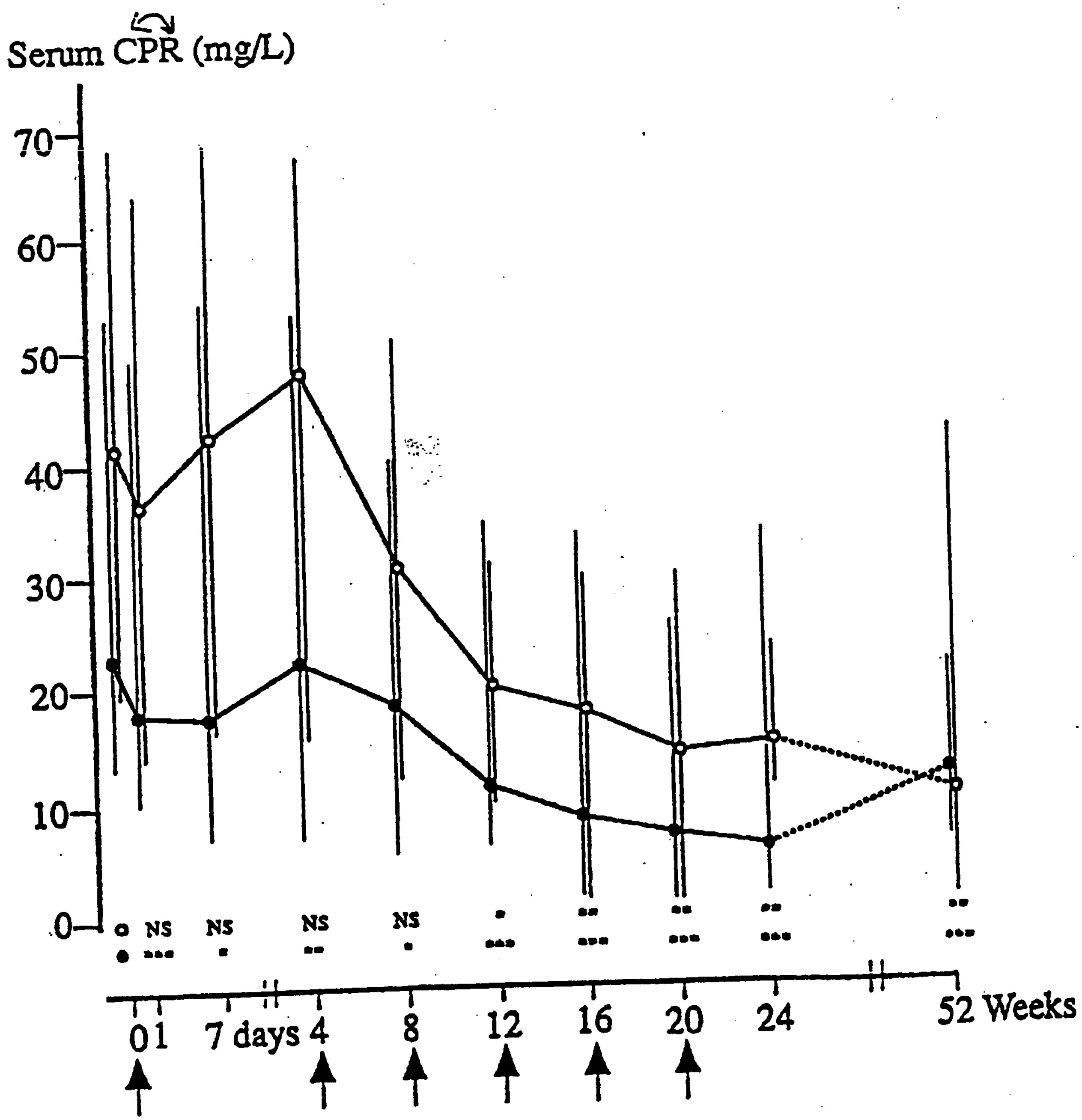

Serum CPR (mg/L)
70
60
50
40
30
20
10
0
NS
NS
NS
NS
0 1
7 days
4
8
12
16
20
24
52 Weeks

FIGURE 5a

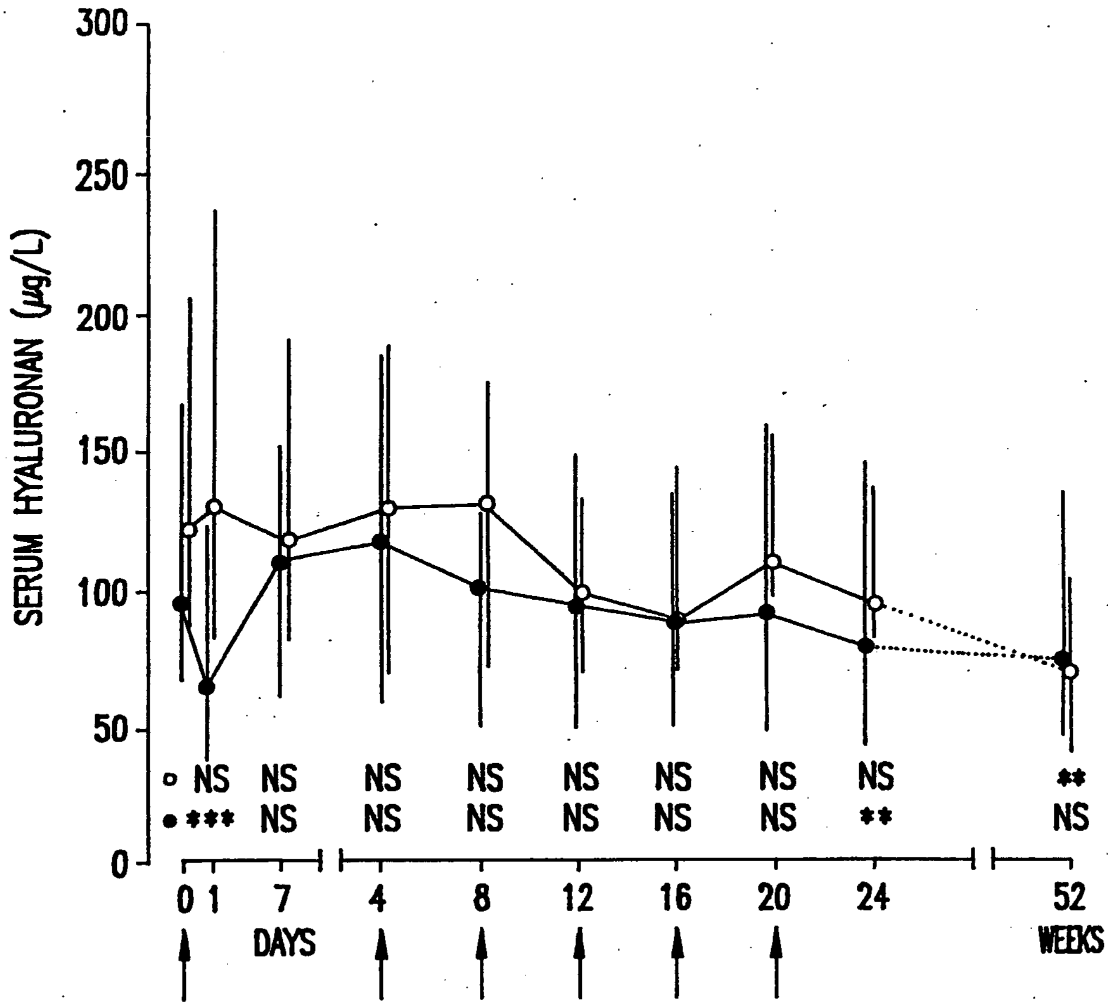
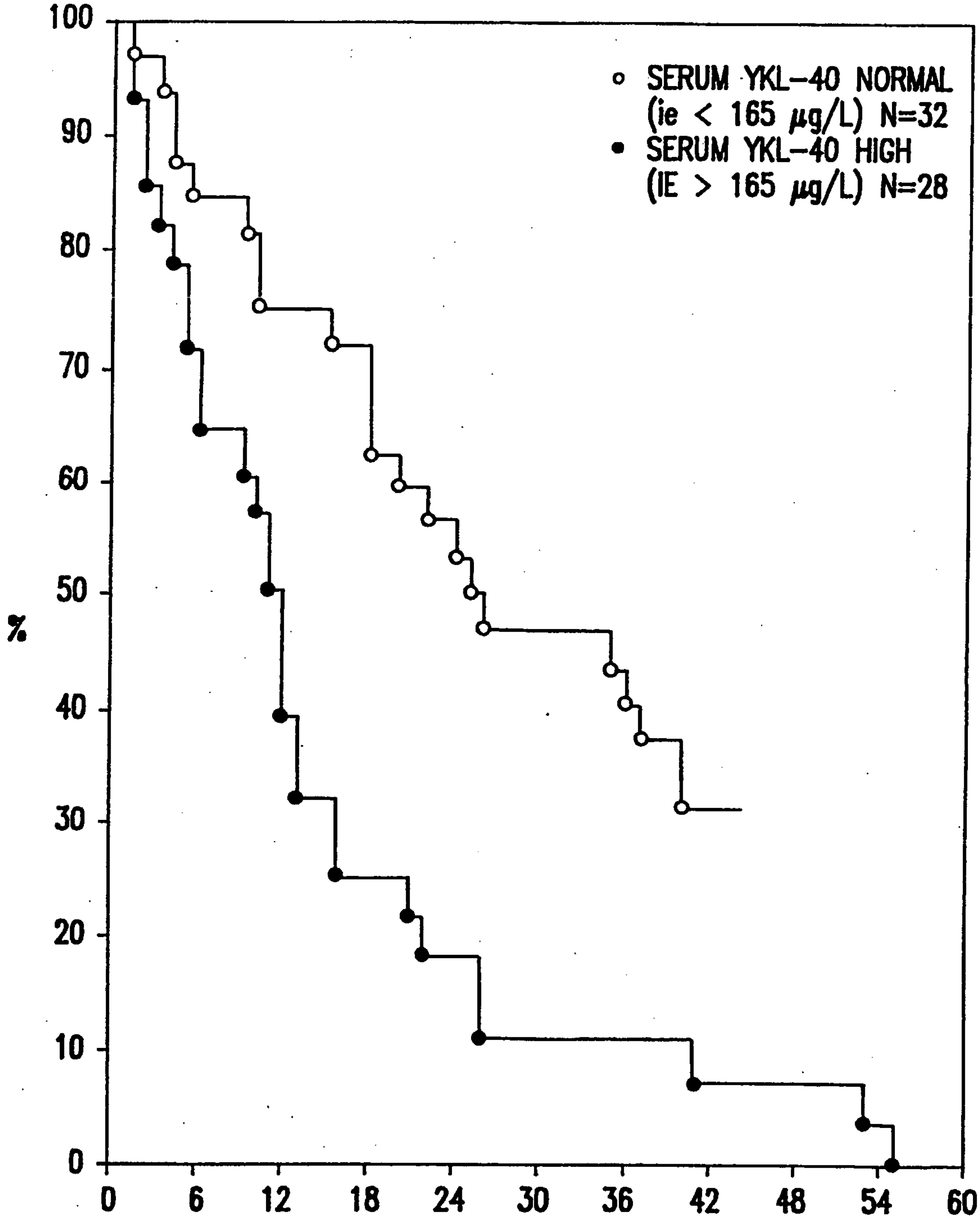

SERUM HYALURONAN (µg/L)
300
250
200
150
100
50
0
○ NS NS NS NS NS NS NS NS **
● *** NS NS NS NS NS NS ** NS
0 1 7
DAYS
4 8 12 16 20 24 52
WEEKS

FIG.5b



SERUM YKL-40 NORMAL (ie < 165 µg/L) N=32
SERUM YKL-40 HIGH (IE > 165 µg/L) N=28
%
100
90
80
70
60
50
40
30
20
10
0
0
6
12
18
24
30
36
42
48
54
60

FIG.6

8/11

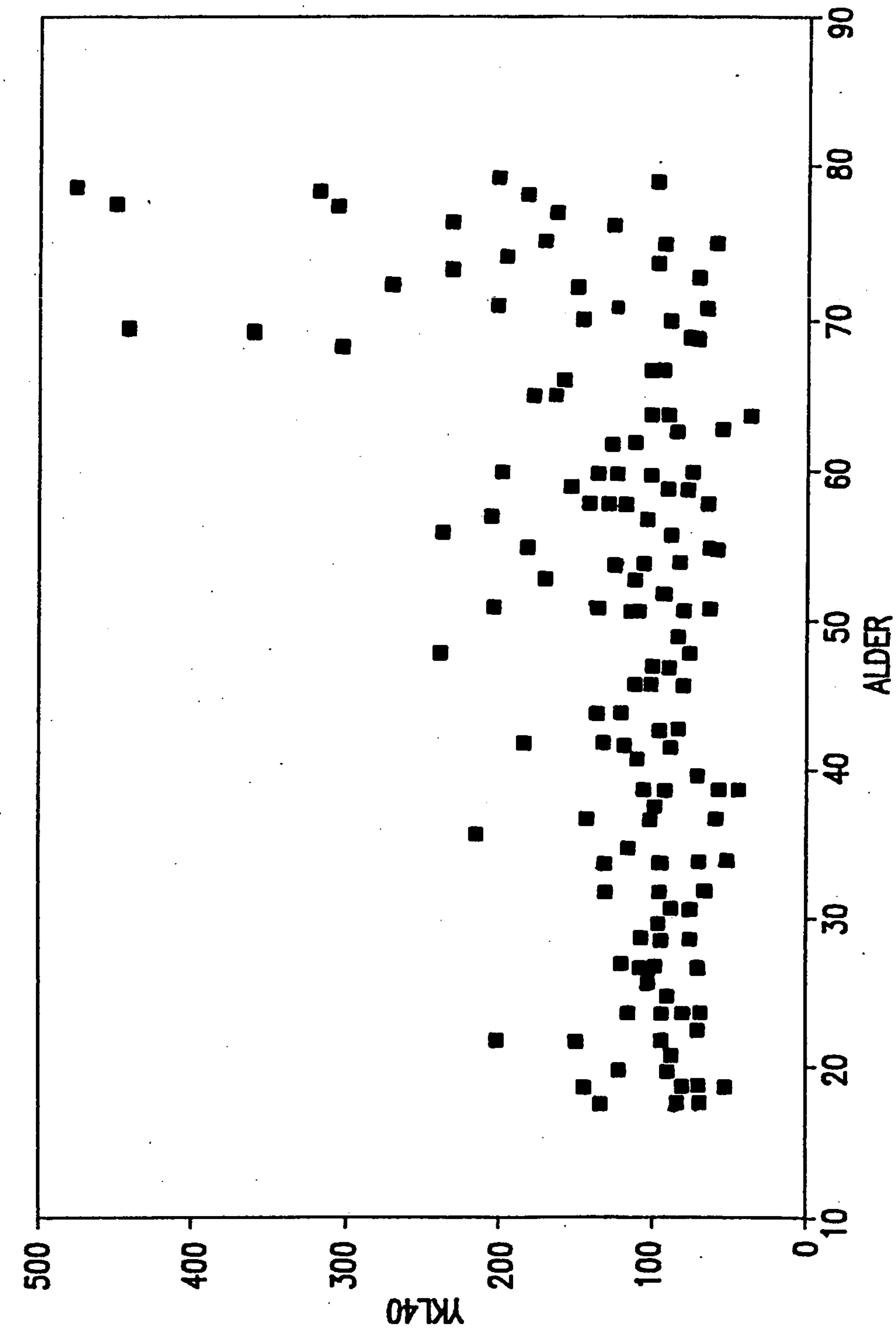
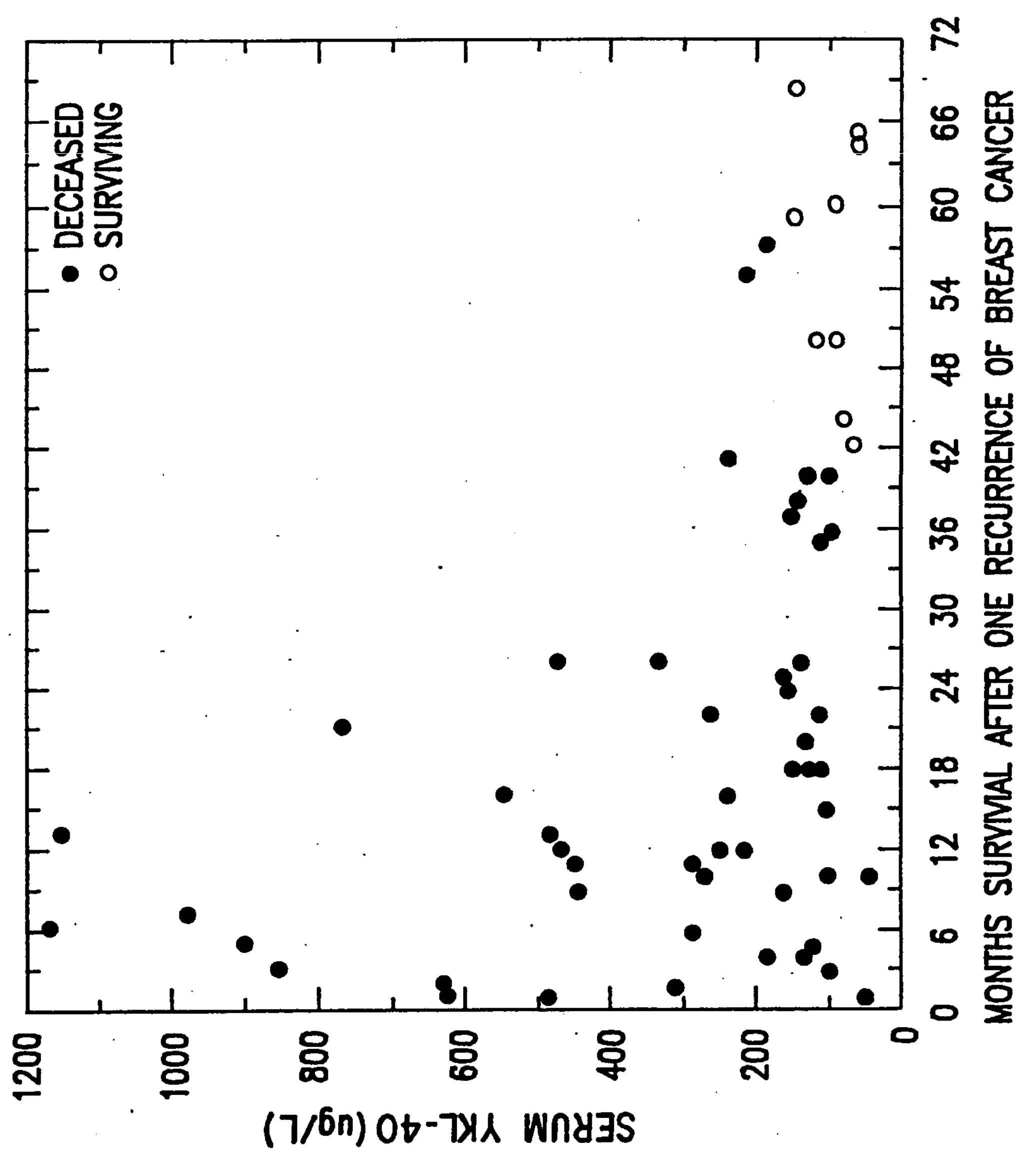
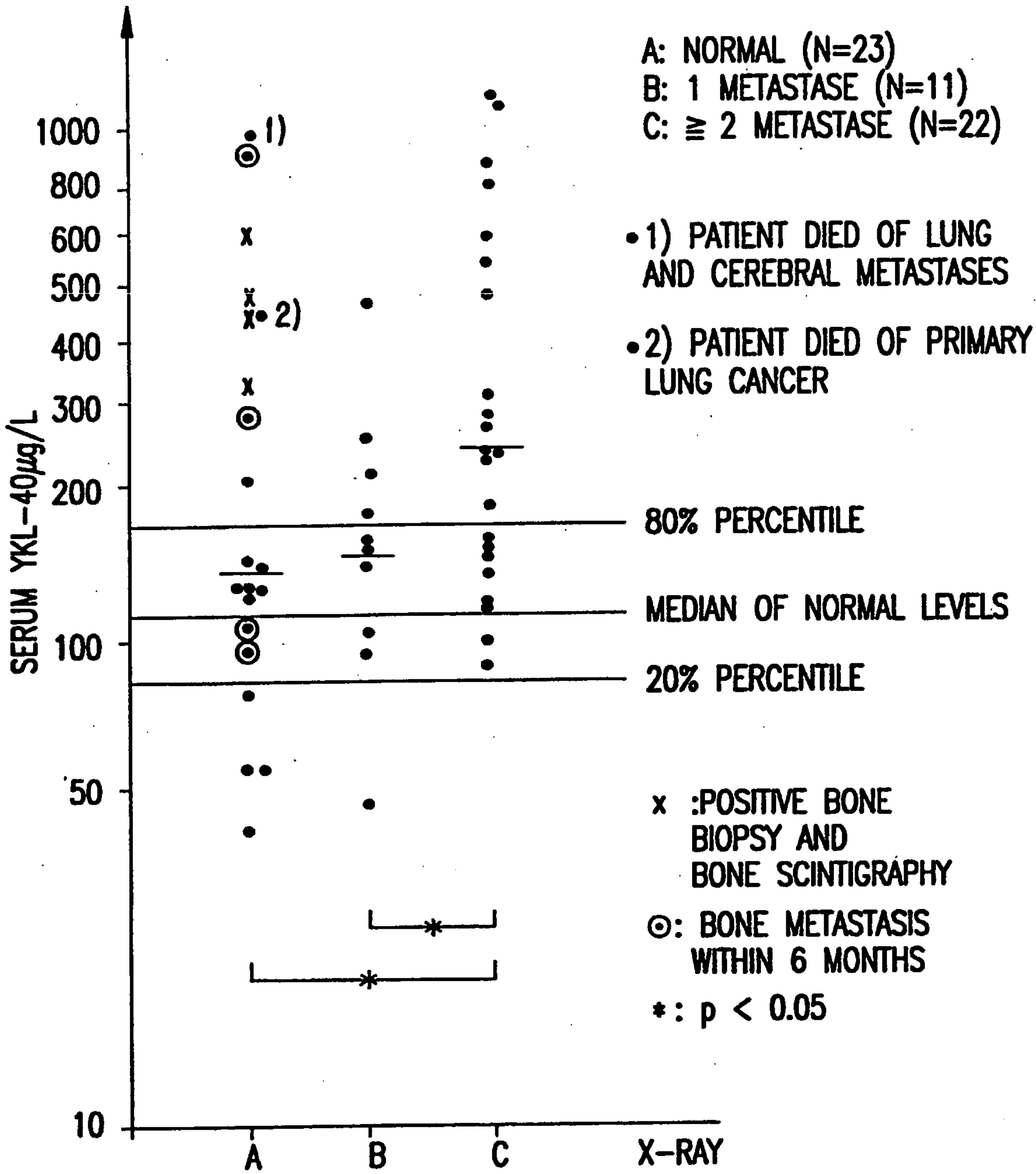


FIG.7

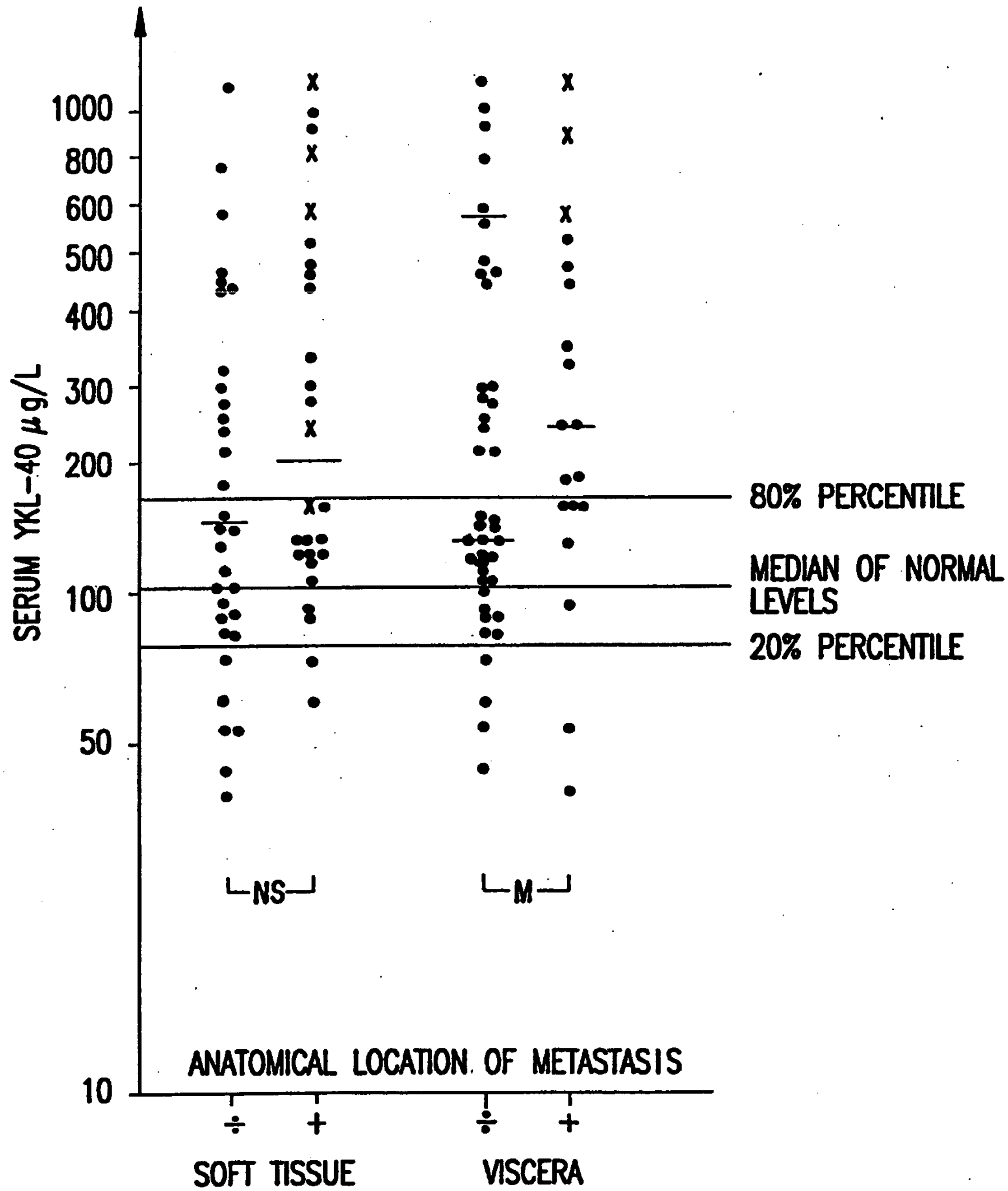
FIG.8


DECEASED
SURVIVING
MONTHS SURVIVAL AFTER ONE RECURRENCE OF BREAST CANCER
SERUM YKL-40 (ug/L)
0
6
12
18
24
30
36
42
48
54
60
66
72
1200
1000
800
600
400
200
0



A: NORMAL (N=23)
B: 1 METASTASE (N=11)
C: ≧ 2 METASTASE (N=22)
1)
2)
• 1) PATIENT DIED OF LUNG AND CEREBRAL METASTASES
• 2) PATIENT DIED OF PRIMARY LUNG CANCER
SERUM YKL-40μg/L
1000
800
600
500
400
300
200
100
50
10
80% PERCENTILE
MEDIAN OF NORMAL LEVELS
20% PERCENTILE
x :POSITIVE BONE BIOPSY AND BONE SCINTIGRAPHY
⊙: BONE METASTASIS WITHIN 6 MONTHS
*: p < 0.05
A
B
C
X-RAY

FIG.9



SERUM YKL-40 µg/L
1000
800
600
500
400
300
200
100
50
10
80% PERCENTILE
MEDIAN OF NORMAL LEVELS
20% PERCENTILE
NS
M
ANATOMICAL LOCATION OF METASTASIS
÷
+
÷
+
SOFT TISSUE
VISCERA

FIG.10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/06579

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :G01N 33/574, 33/53; C07K 15/28
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.23, 7.9, 7.93; 436/64, 813; 530/387.7, 388.1, 388.25, 388.8, 388.85, 389.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG: CAB Abstracts, Biosis, Medline, Embase, Cancerlit, Derwent; Search terms: YKL40, YKL(w)40, Price P A, Johansen J P, connective, bone, matrix, metasta?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J. Bone Miner Res, Volume 7, No. 5, issued May 1992, JS Johansen et al, "Identification of proteins secreted by human osteoblastic cells in culture," pages 501-12, ABSTRACT ONLY, see lines 20-25 of the abstract.	1-26

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
17 October 1993

Date of mailing of the international search report
NOV 02 1993

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/06579

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/7.23, 7.9, 7.93; 436/64, 813; 530/387.7, 388.1, 388.25, 388.8, 388.85, 389.1